

second paragraph. The applicants respectfully traverse the rejections if applied to the claims as amended.

The Examiner asserted that it is unclear what the composition enhances the transport of because the Markush group of claim 1 includes a carrier thereby rendering claim 1 without purpose. The claim has been amended to more clearly define the conjugate as including a pH sensitive polymer and a second unit that comprises a diagnostic, therapeutic, prophylactic agent or carrier. A pH-sensitive polymer conjugated to, complexed with or incorporated with a carrier, can be used to enhance transport of an endogenous material through a lipid-containing membrane. This is sufficient to comply with the utility requirements as defined by MPEP § 2107.

Claim 22 has been amended to recite that the composition of the solution is changed. See page 17, lines 5-11, and references cited therein.

The Examiner further asserted that the term "esters of acrylic acid copolymerized with acrylic acid" recited in claim 28 renders the claim indefinite. The applicants respectfully disagree. To one of ordinary skill in the art, the recited term is definite and refers to copolymers formed by polymerization of esters of acrylic acid and acrylic acid.

The Examiner further alleged that claim 9 was indefinite for reciting "a compound which decreases lysosomal degradation." A compound can decrease lysosomal degradation by decreasing production of acid within the lysosome, or by decreasing the acidity of the lysosome directly. Decreasing production of acid within the lysosome can be achieved by, for example, inhibiting enzymes involved in acid production in lysosome. The Examiner argued that "it is unclear how these compounds enter the lysosomes to decrease the acid production." However,

claim 9 is drawn only to the compounds which are known to decrease lysosomal degradation, such as those listed at page 27, lines 14-16. All are known to be effective and are widely used for the claimed purpose. Other such agents are also known, described in the literature and commercially available. The alleged lack of explanation of how these compounds enter the lysosomes to decrease the acid production or the acidity of the lysosomes, if any, does not render the claim indefinite (see MPEP § 2163.07).

Rejection under 35 U.S.C. § 102

Claims 1, 5, 7, 9, 18-19, 21-22, 28-29 and 33 were rejected as allegedly anticipated under 35 U.S.C. § 102(a) by U.S. Patent No. 5,609,590 to Herbig ("Herbig"). Claims 1, 5, 7-13, and 18-33 were rejected as allegedly anticipated under 35 U.S. C. § 102(a) by WO 97/09068 by the University of Washington ("WO 068"). Claims 1, 5, 7, 9-10, 18-25 and 33 were rejected as allegedly anticipated under 35 U.S.C. § 102(e) by U.S. Patent No. 5,807,306 to Shapland ("Shapland"). Claims 1, 3, 5, 8-13, 17-19, 21-22, 27, 30 and 33 were rejected as allegedly anticipated under 35 U.S.C. § 102(e) by U.S. Patent No. 5,753,263 to Lishko ("Lishko"). The applicants respectfully traverse the rejections if applied to the claims as amended.

*The claimed invention*

The claimed compositions and methods of use thereof are directed to enhanced transport through a cell membrane, cell component membrane or phospholipid membrane by using a pH-sensitive polymer conjugated to a material to be delivered into a cell. The polymer has a higher hydrophobicity or lipophilicity upon protonation and a lower hydrophobicity upon deprotonation. Therefore, the polymer has a better capability to penetrate or disrupt a lipid-

containing membrane upon protonation, and less capability upon deprotonation. The compositions include, for example, (1) a pH-sensitive polymer which does not disrupt cell membranes at physiological pH but which disrupts the endosomal membrane at the pH range inside the endosomes in combination with (2) a second component which includes a carrier, a therapeutic agent, a diagnostic agent or a combination thereof. The second component is conjugated to, complexed with, or incorporated into the first polymer, and the composition optionally can be provided in a carrier such as nanoparticles, microparticles, and liposomes. The ability of the composition to transport through the membrane can be further enhanced by the application of a stimulus means which induces a change in the structure of the polymer of the composition such as a change in pH or a stimulus means such as ultrasound.

*Herbig*

Herbig discloses osmotic bursting devices for dispensing a drug in an aqueous environment (abstract). The devices utilize a pH sensitive material coating a drug capsule such that the pH sensitive material is semipermeable to water and causes hydrostatic pressure within the capsule to build and eventually burst the capsule, or the pH sensitive material is used to hold together two capsule portions until it dissolves or disintegrates in contact with an environment having a particular pH to release the capsule contents (col. 6, lines 40-58). There is no disclosure or suggestion in Herbig, however, of a pH-sensitive polymer which is conjugated to or has incorporated therein a second unit which contains a carrier, a therapeutic or diagnostic agent.

Further, as Figure 1 and Figure 2 of Herbig indicate, Herbig uses the pH-sensitive material as a part of the capsule material, and the drug is in the form of a tablet. Therefore, there

is no binding force whatsoever, i.e., chemical bond or physical interaction, between a molecule of the pH-sensitive material and a drug molecule. Therefore, the molecules of the pH sensitive material described in Herbig, once dissolved in a body **cannot enhance the transport of a drug molecule through a lipid-containing membrane** as the claimed subject matter does. As such, Herbig can not anticipate the claimed subject matter under 35 U.S.C. § 102(a).

*WO 068*

WO 068 discloses stimuli-responsive polymers conjugated to interactive molecules (p. 9, lines 8-23). The stimuli-responsive polymer is responsive to an external stimulus such as a change in solution pH or ionic strength (p. 10, lines 20-23). The interactive molecule is a biomolecule such as a protein or peptide, polysaccharide or glycoprotein which specifically binds to a ligand (p. 22, line 16 to p. 23, line 13). Therefore, the interactive molecules include both ligands and receptors (p. 22, lines 18-19). The function of the interactive molecule is controlled by a change in an external stimuli, such as temperature or pH, and the change cause the stimuli-responsive polymer to undergo a conformational or physico-chemical change which leads to a structural transition at or near or distant to the site of attachment, *thereby modulating the activity of the interactive molecule in the process* (p. 9, lines 9-23), not modulating the activity of another object such as a cell membrane.

The Examiner asserted that the same pH-sensitive polymer is used in WO 068, and thus, the claims were anticipated. However, the applicants respectfully direct the Examiner to Figures 1a-1d and 2a-2d of WO 068. The figures clearly show that, upon application of a stimulus, the stimulus-sensitive polymer undergoes a conformational change so as to switch on or off the

binding of the interactive molecule to a target. Therefore, the critical aspect of WO 068 is not that the stimulus-sensitive polymer in WO 068 enhances transport through a lipid-containing membrane of an interactive molecule but, rather, that the stimulus-sensitive polymer is used to control the binding of the interactive molecule which is conjugated to the stimulus-sensitive polymer to a target. Moreover, the stimulus-sensitive polymer of WO 068 is relatively small (1,000 to 30,000 Daltons, see p. 11, lines 13-14) as compared to the interactive molecule which is a large biomolecule such as antibody or polysaccharide which usually has a molecular weight in the range of hundreds of thousands Daltons. Therefore, the size of the stimulus-sensitive polymer is only a very small fraction of the interactive molecule. As such, to one of ordinary skill in the art, the stimulus-sensitive polymer in WO 068 would not enhance the transport of the interactive molecule conjugate through a lipid-containing membrane.

*Shapland*

Shapland discloses a drug delivery apparatus and method for delivering a drug encapsulated in a polymeric matrix to internal body tissue using a catheter device and iontophoresis or phonophoresis (abstract). However, Shapland does not disclose a pH-sensitive polymer as defined in the claims. Rather, Shapland uses polymers such as polyvinyl alcohol or polyacrylamide (col. 9, lines 26-37) which, to one of ordinary skill in the art, cannot become more or less hydrophobic or hydrophilic upon a pH change from the pH of endosome to the physiological pH. To one of ordinary skill in the art, the R-OH group or RCOO-NR<sub>2</sub> group is not so pH-sensitive so as to render the polymer used in Shapland pH-sensitive under physiological conditions. Therefore, Shapland does not anticipate the claimed subject matter

under 35 U.S.C. § 102(e).

*Lishko*

Lishko discloses encapsulation of compounds in liposomes for targeted delivery to hair follicles (col. 3, lines 19-38). Lishko fails, however, to disclose or suggest a pH-sensitive polymer which becomes more hydrophobic and more lipophilic at a pH between about 5 and 6.5 and which is conjugated to or has incorporated therein a second polymeric or monomeric unit which bonds to a therapeutic or diagnostic agent. Therefore, Lishko fails to disclose the claimed conjugate or methods of use thereof.

The Examiner asserted that Lishko teaches pH-sensitive polymer at col. 15, line 13 to col. 20. In contrast, at col. 18, lines 51-64, teaches that the beneficial compound can be a macromolecule or polymer which is too large to penetrate stratum corneum or lipid barriers. Therefore, Lishko does not disclose any pH-sensitive polymer as asserted by the Examiner. Further, the polymer disclosed in Lishko is encapsulated within pH-sensitive liposomes for delivery to hair follicles. This fact clearly shows that the polymer disclosed in Lishko is not itself capable of penetrating or disrupting a lipid-membrane upon a change of pH. As such, Lishko does not anticipate the claims under 35 U.S.C. § 102(e).

Rejection under 35 U.S.C. § 103

Claims 1, 5, 8-13, 17-19, 21-22, 27-31 and 33 were rejected under 35 U.S.C. § 103(a) as obvious over Lishko. The applicants respectfully disagree if applied to the claims as amended.

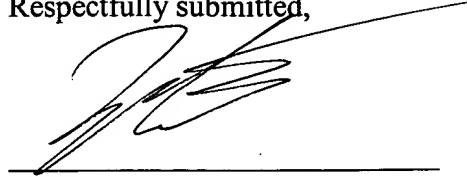
As discussed above, Lishko fails to teach or suggest (1) a pH-sensitive polymer, which is critical in the claimed subject matter, and further (2) the pH-sensitive polymer, together with a

second unit which includes a carrier, a therapeutic agent, or a diagnostic agent, or a combination therefore having an enhanced transport through a lipid-containing membrane when the pH changes from the physiological pH to the pH of endosomes. Moreover, Lishko is drawn to targeted delivery of liposome-encapsulated compounds to hair follicles. Therefore, Lishko does not provide motivation for one of ordinary skill in the art to modify Lishko so as to make and use a composition which has an enhanced transport through a lipid-containing membrane when the pH changes from the physiological pH to the pH of endosomes. Even if one argued that Lishko provides a motivation for one of ordinary skill in the art to make and use the claimed subject matter, one of ordinary skill in the art would appreciate that the teachings of targeted delivery of an liposome encapsulated compound to hair follicles may not be applied to making and using a composition that has an enhanced transport through a lipid-containing membrane when the pH changes from the physiological pH to the pH of endosomes. Therefore, there is no reasonable expectation of success. As such, Lishko does not render claims 1, 5, 8-13, 17-19, 21-22, 27-31 and 33 *prima facie* obvious under 35 U.S.C. § 103.

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AMENDMENT AND RESPONSE TO OFFICE ACTION

Applicants therefore respectfully request allowance of claims 1, 5, 7-13, and 17-33, as amended.

Respectfully submitted,

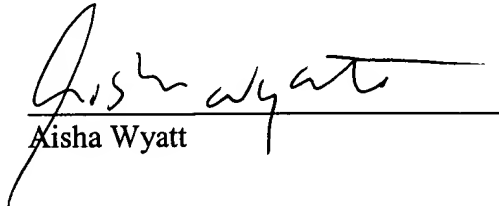


Zhaoyang Li  
Reg. No. 46,872

Date: January 3, 2002  
Holland & Knight LLP  
One Atlantic Center Suite 2000  
1201 W. Peachtree Street  
Atlanta, GA 30309-3400  
(404) 817-8473  
(404) 817-8588 fax  
ppabst@hklaw.com

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Date: January 3, 2002

**APPENDIX: Marked Copy of Claims as Amended**

1. (four times amended) A polymeric composition for enhancing transport through a cell membrane, cell component membrane or phospholipid membrane comprising

a first pH-sensitive polymer which is not hydrophobic at a first pH, but which is more hydrophobic and more lipophilic and thereby enhances transport through the cell membrane, cell component membrane, liposome or lipid vesicle at a second pH,

a second unit conjugated to, complexed with, or incorporated with the first pH- sensitive polymer, wherein the unit comprises a material [is] selected from the group consisting of a carrier, a therapeutic agent, a diagnostic agent, and combinations thereof.

5. (three times amended) The composition of claim 1 wherein the second unit comprises a therapeutic or diagnostic agent, the composition further comprising a pharmaceutically acceptable carrier.

7. (four times amended) The composition of claim 1 wherein the second unit comprises a polymer and the first pH-sensitive polymer and the second unit form a graft copolymer, block copolymer, random copolymer or blend thereof.

8. (twice amended) The composition of claim 1 wherein the second unit is linked to a ligand binding to the surface of a cell.

9. The composition of claim 1 further comprising a compound which decreases lysosomal degradation.

10. The composition of claim 5 wherein the therapeutic agent is a cytotoxic compound.

11. (twice amended) The composition of claim 1 wherein the second unit comprises a

polycationic polymer or cationic lipid.

12. (Once amended) The composition of claim 5 wherein the therapeutic agent is a nucleoside, nucleotide, or nucleic acid.

13. (Once amended) The composition of claim 1 further comprising a carrier selected from the group consisting of microparticles, nanoparticles, micelles and liposomes.

17. (amended) The method of claim 33 wherein the composition is administered to cells in a suspension.

18. (amended) The method of claim 33 wherein the composition is administered to layers of cells to enhance transport through the cell layers.

19. (amended) The method of claim 33 wherein the composition is administered to liposomes or lipid vesicles to enhance transport of molecules into or out of the liposomes or lipid vesicles.

20. (three times amended) The method of claim 33 wherein the composition is administered in combination with electrophoresis or iontophoresis.

21. (twice amended) The method of claim 33 further comprising application of a stimulus means to further enhance the effectiveness of the composition to alter transport through the membrane, wherein the stimulus means induces a change in the structure of the polymer of the composition.

22. (three times amended) The method of claim 21 wherein the stimulus means is selected from the group consisting of changes in pH, light, ionic strength, [solvent to alter solubility of the] changes in solution composition, temperature, and electric field.

23. (amended) The method of claim 33 further comprising administration of a stimulus means to further enhance the effectiveness of the composition to alter transport through the membrane, wherein the stimulus means is selected from the group consisting of ultrasound, electrical fields, radiation, and combinations thereof.

24. The method of claim 23 wherein the stimulus means is ultrasound.

25. The method of claim 24 wherein the ultrasound is administered at between 20 kHz and 10 MHz.

26. (twice amended) The composition of claim 11 wherein the polycationic material is selected from the group consisting of chitosan, polylysine, polyethyleneimine, poly(propyleneimine), aminodextran, collagen, polyvinylimidazole, and N,N-dimethylaminoethyl methylacrylate.

27. The composition of claim 13 wherein the carrier is a micelle or liposome.

28. (twice amended) The composition of claim 7 wherein the pH sensitive polymer is selected from the group consisting of acrylic acid polymers; C<sub>1-6</sub> straight chain, branched, ethylene-acrylic acid copolymers and cyclic 2-alpha-alkyl acrylic acids; vinyl imidazole polymers and esters of acrylic acid copolymerized with acrylic acid.

29. (twice amended) The composition of claim 7 wherein the second unit comprises polymeric blocks comprising proteins or peptides which include imidazole groups.

30. (amended) The composition of claim 1 wherein the second unit comprises a lipid or phospholipid.

31. (amended) The composition of claim 1 wherein the second unit comprises

sulfonated groups.

32. (amended) The composition of claim 1 wherein the second unit is sensitive to a stimulus selected from the group consisting of temperature, light, electrical stimuli, radiation, pH and ion concentration.

33. A method for enhancing transport of agents through cell membranes, cell component membranes or liposomes or lipid vesicles comprising administering to the cell membrane cell component membrane, liposome or lipid vesicle any of the compositions of claims 1, 5, 7-13, and 26-32.

**Appendix: Clean Copy of Claims as Amended**

1. (four times amended) A polymeric composition for enhancing transport through a cell membrane, cell component membrane or phospholipid membrane comprising

a first pH-sensitive polymer which is not hydrophobic at a first pH, but which is more hydrophobic and more lipophilic and thereby enhances transport through the cell membrane, cell component membrane, liposome or lipid vesicle at a second pH,

a second unit conjugated to, complexed with, or incorporated with the first pH- sensitive polymer, wherein the unit comprises a material selected from the group consisting of a carrier, a therapeutic agent, a diagnostic agent, and combinations thereof.

5. (three times amended) The composition of claim 1 wherein the second unit comprises a therapeutic or diagnostic agent, the composition further comprising a pharmaceutically acceptable carrier.

7. (four times amended) The composition of claim 1 wherein the second unit comprises a polymer and the first pH-sensitive polymer and the second unit form a graft copolymer, block copolymer, random copolymer or blend thereof.

8. (twice amended) The composition of claim 1 wherein the second unit is linked to a ligand binding to the surface of a cell.

9. The composition of claim 1 further comprising a compound which decreases lysosomal degradation.

10. The composition of claim 5 wherein the therapeutic agent is a cytotoxic compound.

11. (twice amended) The composition of claim 1 wherein the second unit comprises a

polycationic polymer or cationic lipid.

12. (Once amended) The composition of claim 5 wherein the therapeutic agent is a nucleoside, nucleotide, or nucleic acid.

13. (Once amended) The composition of claim 1 further comprising a carrier selected from the group consisting of microparticles, nanoparticles, micelles and liposomes.

17. (amended) The method of claim 33 wherein the composition is administered to cells in a suspension.

18. (amended) The method of claim 33 wherein the composition is administered to layers of cells to enhance transport through the cell layers.

19. (amended) The method of claim 33 wherein the composition is administered to liposomes or lipid vesicles to enhance transport of molecules into or out of the liposomes or lipid vesicles.

20. (three times amended) The method of claim 33 wherein the composition is administered in combination with electrophoresis or iontophoresis.

21. (twice amended) The method of claim 33 further comprising application of a stimulus means to further enhance the effectiveness of the composition to alter transport through the membrane, wherein the stimulus means induces a change in the structure of the polymer of the composition.

22. (three times amended) The method of claim 21 wherein the stimulus means is selected from the group consisting of changes in pH, light, ionic strength, changes in solution composition, temperature, and electric field.

23. (amended) The method of claim 33 further comprising administration of a stimulus means to further enhance the effectiveness of the composition to alter transport through the membrane, wherein the stimulus means is selected from the group consisting of ultrasound, electrical fields, radiation, and combinations thereof.

24. The method of claim 23 wherein the stimulus means is ultrasound.

25. The method of claim 24 wherein the ultrasound is administered at between 20 kHz and 10 MHz.

26. (twice amended) The composition of claim 11 wherein the polycationic material is selected from the group consisting of chitosan, polylysine, polyethyleneimine, poly(propyleneimine), aminodextran, collagen, polyvinylimidazole, and N,N-dimethylaminoethyl methylacrylate.

27. The composition of claim 13 wherein the carrier is a micelle or liposome.

28. (twice amended) The composition of claim 7 wherein the pH sensitive polymer is selected from the group consisting of acrylic acid polymers; C<sub>1-6</sub> straight chain, branched, ethylene-acrylic acid copolymers and cyclic 2- $\alpha$ -alkyl acrylic acids; vinyl imidazole polymers and esters of acrylic acid copolymerized with acrylic acid.

29. (twice amended) The composition of claim 7 wherein the second unit comprises polymeric blocks comprising proteins or peptides which include imidazole groups.

30. (amended) The composition of claim 1 wherein the second unit comprises a lipid or phospholipid.

31. (amended) The composition of claim 1 wherein the second unit comprises

sulfonated groups.

32. (amended) The composition of claim 1 wherein the second unit is sensitive to a stimulus selected from the group consisting of temperature, light, electrical stimuli, radiation, pH and ion concentration.

33. A method for enhancing transport of agents through cell membranes, cell component membranes or liposomes or lipid vesicles comprising administering to the cell membrane cell component membrane, liposome or lipid vesicle any of the compositions of claims 1, 5, 7-13, and 26-32.

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